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Review

Exploring the accessible conformations of N-terminal acetylated α-synuclein

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ABSTRACT

Alpha synuclein (αsyn) fibrils are found in the Lewy Bodies of patients with Parkinson's disease (PD). The aggregation of the αsyn monomer to soluble oligomers and insoluble fibril aggregates is believed to be one of the causes of PD. Recently, the view of the native state of αsyn as a monomeric ensemble was challenged by a report suggesting that αsyn exists in its native state as a helical tetramer. This review reports on our current understanding of αsyn within the context of these recent developments and describes the work performed by a number of groups to address the monomer/tetramer debate. A number of in depth studies have subsequently shown that both non-acetylated and acetylated αsyn purified under mild conditions are primarily monomer. A description of the accessible states of acetylated αsyn monomer and the ability of αsyn to self-associate is explored.

1. Introduction

Parkinson's disease (PD) research has sought to answer questions of alpha synuclein (αsyn) function and the mechanism of aggregation surrounding disease pathology. Both remain to be fully articulated today, but several observations have been established and a range of neurodegenerative diseases termed the "synucleinopathies" have been identified [1,2]. PD in particular is the synucleinopathy characterized by the loss of dopaminergic neurons and is largely considered to be an age-related disease, accompanied in part by age-related deposition of αsyn [3]. αsyn, a major protein component of Lewy Bodies [4,5] in patients with Parkinson's, is a small primarily neuronal protein that is known to make a structural transition to amyloid fibrils [6–8]. αsyn is expressed abundantly in the nervous system and localizes near presynaptic nerve terminals [9–13]. It is also expressed at high levels in erythrocytes and platelets [14]. αsyn's function is unknown, but there is strong evidence that it exhibits lipid binding in vesicles and synaptic membranes [15] and may somehow exert its pathology through this behavior [16]. There is evidence that αsyn functions in assembly of the SNARE complex involved in vesicle transport [17], that it may more generally be involved in synaptic vesicle trafficking and regulation and/or may play a key role in neuronal cell survival [18–22].

The deposition of αsyn has largely been thought to originate from an intrinsically disordered monomer ensemble that under fibril promoting conditions forms amyloid [7,23,24], but recently this view of αsyn's native state was challenged [25]. Selkoe and colleagues pushed the biophysical community's long-held view of αsyn as an intrinsically disordered monomer by suggesting that the protein exists in its native state as a fibril-resistant helical tetramer. They purified the sample from human erythrocytes, opting to exclude a potentially "harsh" and commonly used boiling step

Abbreviations: αsyn, α-synuclein; Ac-αsyn, acetylated α-synuclein; BOG, beta-octyl glucopyranoside; GST, glutathione S-transferase; CD, circular dichroism; CN-PAGE, clear native PAGE; ESI-MS, electrospray ionization-mass spectrometry; ESI–IMS-MS, electrospray ionization–ion mobility spectrometry-mass spectrometry; ELISA, enzyme-linked immunosorbent assay; IDP, intrinsically disordered protein; Nat, N-acetyltransferase; NatB, N-acetyltransferase B; NAC, non-amyloid component region; NMR, nuclear magnetic resonance; PD, Parkinson's disease; PTM, post-translational modifications; RBC, red blood cells; SE-AUC, sedimentation equilibrium-analytical ultracentrifugation; SEC, size exclusion chromatography; SLS, static light scattering; ThT, thioflavin T

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from the purification. Based on this work several questions presented themselves. Do bacterial systems that are commonly used to obtain sample for biophysical characterization not possess the necessary machinery for tetramer assembly? Could the commonly used boiling step during purification denature some key native structure that promoted a helical tetramer of αsyn? Aside from these assembly and purification issues, there was also one molecular difference between the purified samples of Selkoe and colleagues and previous studies, indicative of modification to the monomer by an acetyl group (Ac-αsyn).

This review reports on our current understanding of αsyn within the context of these recent developments and describes the work performed by a number of groups to address the monomer/tetramer debate [25–33]. We summarize major shifts within recently published works addressing these issues in Table 1. Numerous studies indicate that αsyn, both acetylated and non-acetylated, exists as intrinsically disordered monomer conformational ensemble under mild purification conditions. We highlight that the ensemble of monomers is known to develop into a wide range of accessible conformations upon changes of environmental conditions, that it can populate many soluble oligomeric states of varying morphologies and toxicities, and settle into various insoluble fibril or amorphous aggregate morphologies [23], that have largely been studied in the context of PD-related pathology (Fig. 1). We discuss the suggestion of a soluble fibril-resistant helical tetramer that presumably represents a non-pathological aggregate of αsyn which may have to dissociate before fibril formation can proceed through the monomer (Fig. 1). The potential that established methods might disrupt native-stabilizing interactions of a fibril-resistant helical tetramer of αsyn have heightened awareness to cell machinery, to αsyn purification methods, and to the difficulties in choosing appropriate methods of characterization. The extent to which N-terminal acetylation impacts upon the conformation and aggregation behavior of αsyn is discussed separately and it is shown that the acetyl group does not promote the formation of the helical tetramer under mild purification conditions.

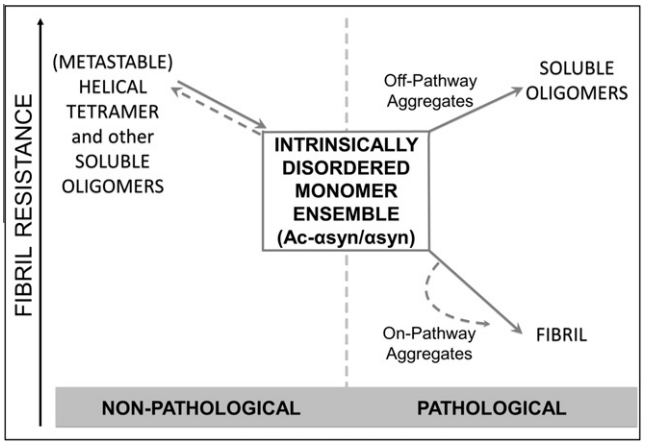

Fig. 1. A schematic diagram of the possible accessible states of non-acetylated and acetylated αsyn. The right side represents two possible pathological aggregation pathways from the unfolded monomeric ensemble to (1) insoluble fibrils through on-pathway transient oligomeric intermediates and (2) to off-pathway soluble oligomers. Off-pathway soluble oligomers represent non-fibrillar end products of aggregation. The left side presents (1) the recent proposal that αsyn can exist as a soluble fibril-resistant helical tetramer which is acetylated, and (2) other known oligomers that are not toxic such as methionine oxidized oligomers. It is proposed that the non-pathological tetramer needs to dissociate to the monomeric ensemble before pathological aggregation can occur (solid arrow). The relationship between the unfolded monomeric ensemble and the proposed tetramer is a subject of investigation (dashed arrow).

2. Overview of non-acetylated αsyn ensemble: monomers and dimers

2.1. Biophysical characterization of the non-acetylated monomer ensemble

The native state of non-acetylated αsyn has been thought to originate from an ensemble of intrinsically disordered monomeric forms, with recognition that the monomers therein are capable of adopting a wide range of accessible conformations depending on solution and environmental conditions [34–38]. Uversky first spoke of αsyn as the "protein chameleon" [23] due to its ability to respond to its environment and binding partners by varying its foldedness and aggregation state. αsyn is often described as a 140 residue intrinsically disordered protein (IDP) characterized by three distinct regions of the protein: an N-terminal lipid binding repeat region that houses the mutations A30P, E46K, and A53T linked to early onset disease, a hydrophobic non-amyloid component (NAC) region implicated in fibril formation, and an acidic more proline-rich C-terminus suggested to have chaperone activity and possess some key role in modulating structure in the N-terminus [6,39,40]. As summarized in Table 1, to study PD related aggregation, αsyn has typically been obtained from overexpression in bacteria, yielding a non-acetylated IDP, as bacteria typically do not modify their proteins by acetylation (Fig. 2A) [41–43]. Additionally, while boiling as part of the purification protocol would typically be considered to be harsh for a globular protein, IDP's are in general characterized by thermostability [34]. Because of this heat stability, αsyn has often been boiled to achieve purity. In addition, IDP's like αsyn are generally characterized by a highly charged sequence, a lack of stable secondary structure, and a larger than expected Stokes radius compared to spherical and folded proteins of the corresponding molecular weight [34,44,45].

The αsyn monomer is both unfolded and extended, as it was first reported to have a larger Stokes radius than expected for globular protein of similar molecular weight and a primarily random coil circular dichroism (CD) spectrum [34,36,46]. However, the protein is not fully extended for a protein of its size, implying a slight compaction of the monomeric ensemble [36,47]. Evidence

Table 1
Historical description of shifts in αsyn purification approaches and conformational properties.

	1996–Dec. 2011	>Dec 2011	>May 2012
Source	Mostly bacterial	Mammalian	Bacterial/mammalian
N-terminal acetylation	No	Yes	Yes
Purification protocol	Often denaturing	Non-denaturing	Denaturing and non-denaturing
Average secondary structure	Primarily random coil	Primarily helical	Primarily random coil
Transient initiating N-terminal helix	No	–	Yes
Primary native state	Monomer	Tetramer	Primarily monomer
Fibril prone	Yes	No	Yes
Referring section within text	2–3	5, 7*	6, 8

* The most recent report by Selkoe and colleagues, suggests "metastability" of the tetramer (Section 7).